Synthesis of Hydroxylated Cyclohexenyl- and Cyclohexanyladenines as Potential Inhibitors of S-Adenosylhomocysteine Hydrolase

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(Dihydroxycyclohexenyl)- and (trihydroxycyclohexenyl)adenines and (dihydroxycyclohexanyl)- and (trihydroxycyclohexanyl)adenines were prepared regio- and diastereoselectively by starting from *cis*-3,5-cyclohexadiene-1,2-diol and 1,3-cyclohexadiene. Palladium(0) [Pd(0)]-catalyzed addition of adenine to allylic epoxide **6**, prepared from *cis*-1,2-(isopropylidenedioxy)cyclohexa-3,5-diene, afforded a single product which was chemically and spectroscopically identified as the 1,2-cis addition product **9**. In contrast, treatment of allylic epoxide **6** with adenine in the absence of a Pd(0) catalyst afforded the trans-1,2-ring-opened product **23**. Both **9** and **23** were converted to various di- and trihydroxylated cyclohexenyl- and cyclohexanyladenines. Cyclohexadiene was exploited to obtain related carbocyclic "nucleosides". Monoepoxidation followed by Pd(0)-catalyzed addition of adenine afforded the cis-1,4-addition product **27**. OsO_4 oxidation following by standard methodology yielded **3** and **4**, six-membered ring homologs of carbocyclic nucleosides **1** and **2**, previously shown to be selective and potent inhibitors of *S*-adenosylhomocysteine hydrolase and broad-spectrum antiviral agents. Toward the cyclohexenyladenines, (diethylamino)sulfur trifluoride (DAST) was utilized to effect an unexpected dehydration. All of the hydroxylated cyclohexenyladenine analogs (**10**, **15**, **22**, and **24**) and hydroxylated cyclohexanyladenine analogs (**4**, **11**, **16**, **25**, and **28**) except analog **3** were shown to be devoid of inhibitory effects against bovine liver *S*-adenosylhomocysteine (AdoHcy) hydrolase at concentrations up to 10 μM. Analog **3** showed some inhibitor activity of the hydrolase (1 μM, 26.7%; 10 μM, 59.6%), but it was not sufficient to warrant additional biological evaluation.

In recent years, naturally occurring carbocyclic nucleosides (e.g., neplanocin A,[1] NpcA, and aristeromycin, Ari[2]) and synthetic carbocyclic nucleosides [e.g., 9-(*trans*-2′,-trans-3′-dihydroxycyclopent-4′-enyl)adenine, **1**;[3a-d] Figure 1] have been of interest as broad-spectrum antiviral agents.[4] The antiviral activity of these carbocyclic nucleosides has been correlated with their inhibitory action on the cellular enzyme *S*-adenosylhomocysteine (AdoHcy) hydrolase[5] and their ability to elevate cellular levels of AdoHcy.[6] This elevated cellular level of AdoHcy appears to cause inhibition of mRNA methyltransferases crucial for viral replication.[4f,7]

NpcA and Ari, like many other first generation AdoHcy hydrolase inhibitors, exhibit considerable cellular toxicity, which limits their use as antiviral agents.[4d-i] The cytotoxicity of these naturally occurring carbocyclic nucleosides appears in part to be related to their ability to serve as substrates for cellular kinases,[5] resulting in the formation of carbocyclic nucleotides that have been implicated in cellular toxicity.[4f,8] In earlier studies, our laboratory has shown that the cytotoxicity of NpcA and Ari could be reduced substantially by preparing analogs in which the 4′-hydroxymethyl group was modified to eliminate the possibility of phosphorylation by cellular kinases.[3] These analogs, which include **1** and **2** (Figure 1), are potent inhibitors of AdoHcy hydrolase and potent antiviral agents with reduced cytotoxicity compared to NpcA and Ari.[3,4f-i]

On the basis of these observations, our laboratory undertook the synthesis of the cyclohexenyl- and cyclohexanyladenine nucleosides **3** and **4** (Figure 1) which are homologs of **1** and **2**, respectively. A literature survey revealed that minimal effort has been made to synthesize six-membered carbocyclic nucleosides.[9] In this article, the syntheses of several hydroxylated cyclohexenyl- and cyclohexanyladenines, including **3** and **4**, are described as starting from 1,3-cyclohexadiene and *cis*-3,5-cyclohexadiene-1,2-diol as commercially available synthetic precursors.

Results and Discussion

Chemistry. The synthesis of the desired compounds **3** and **4** was originally envisioned from compound **5**, which

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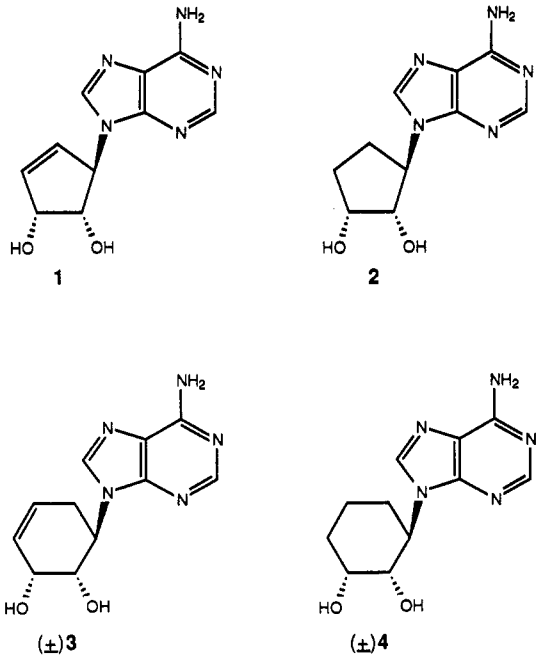
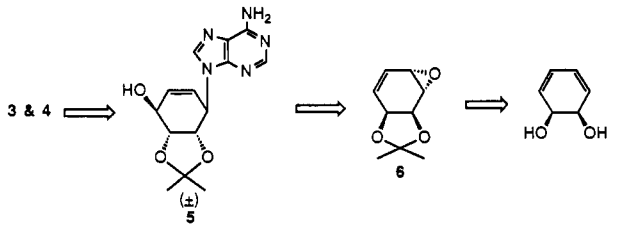
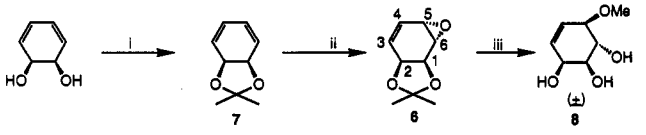


Figure 1.

Scheme I



Scheme II[a]

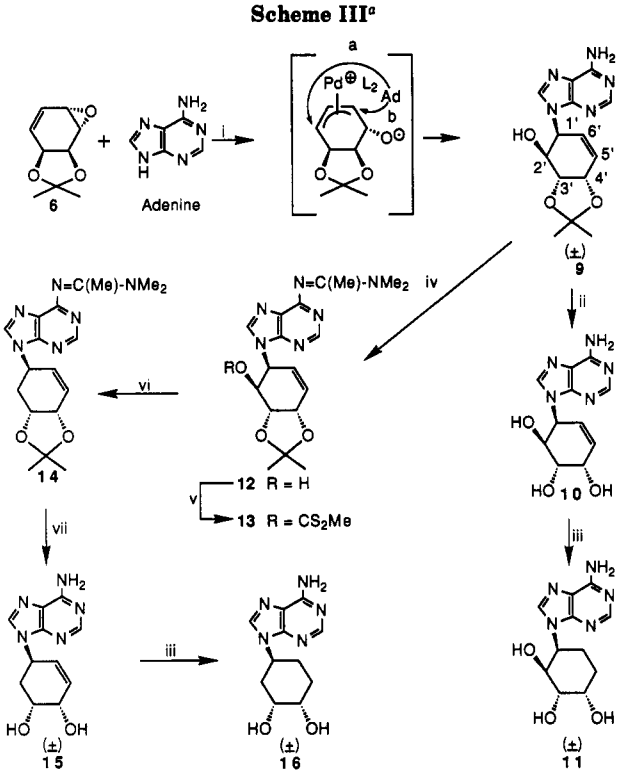


[a] Reagents: (i) 2,2-dimethoxypropane, *p*-TsOH, −15 °C; (ii) *m*-CPBA, CH_2Cl_2, 0 °C; (iii) *d*-10-camphorsulfonic acid, MeOH, CH_2Cl_2.

in turn could be obtained via a nucleophilic addition of adenine to the allylic epoxide **6** catalyzed by palladium(0) [Pd(0)] (Scheme I). A similar reaction has recently been successfully utilized for the synthesis of (±)-Ari from a cyclopentadiene monoepoxide.[10]

To evaluate this possible synthetic route to **3** and **4**, *cis*-3,5-cyclohexadiene-1,2-diol was converted to the isopropylidene derivative **7** by treatment with dimethoxypropane (DMP) and a catalytic amount of *p*-toluenesulfonic acid (*p*-TSOH) at −15 °C (Scheme II). Selective epoxidation of **7** with *m*-chloroperbenzoic acid (*m*-CPBA) in CH_2Cl_2 at 0 °C afforded the desired epoxide **6** in 80% yield (Scheme II). The structure of epoxide **6** was confirmed by comparison of its spectral properties to literature data[11] and by treatment with *d*-10-camphorsulfonic acid

Scheme III[a]



[a] Reagents: (i) $[(i\text{-}C_3H_7O)_3P]_4Pd$, THF, DMSO, 16 h; (ii) (a) aqueous HCl, (b) Dowex-50W (H^+); (iii) Pd–C/H_2; (iv) *N*,*N*-dimethylacetamide dimethyl acetal, MeOH, DMSO, reflux; (v) (a) *n*-BuLi, THF, (b) CS_2, (c) MeI; (vi) Bu_3SnH, AIBN, dioxane, reflux; (vii) (a) aqueous NH_4OH, (b) aqueous HCl, (c) Dowex-50W (H^+).

in MeOH to compound **8**[11] (Scheme II).

Nucleophilic addition of adenine to the monoepoxide **6** (1:1) in the presence of tetrakis(triisopropyl phosphate)–Pd ($[(i\text{-}C_3H_7O)_3P]_4Pd$) as the catalyst generated in situ yielded exclusively one product in 90% yield. The assignment of the structure of the product as **9** via path b as opposed to the expected path a[10] was based on analysis of chemical and spectral data. The ^{13}C-NMR chemical shift values of the purine carbon atoms of the product supported the assignment of the N^9-substitution pattern.[12] However, the ^{13}C- and ^1H-NMR spectra of the product did not unequivocally differentiate between the 1,4-addition product **5** and the 1,2-addition product **9**.

The COSY spectrum (see the supplementary material) shows the sole free hydroxyl proton to be coupled only to the most upfield proton in the carbocyclic ring (at δ 4.14), which was assigned to be at the 2′-position on the basis of chemical shifts and connectivity patterns. The structural assignment of **9** was further confirmed through NOE experiments (see the supplementary material). Irradiation of the signal for the 1′-proton enhanced the signal for the 2′-proton and the 6′-proton. Irradiation of the signal for the 2′-proton enhanced only the signal for the 1′-proton, clearly indicating a cis relationship between these protons. Similarly, irradiation of the signal for the 3′-proton enhanced the signal for the 4′-proton and vice versa, suggesting a cis relationship between these protons.

To further confirm the structural assignment of **9** and to use **9** to generate novel (trihydroxycyclohexenyl)- and (dihydroxycyclohexenyl)adenines and (trihydroxycyclohexanyl)- and (dihydroxycyclohexanyl)adenines, the

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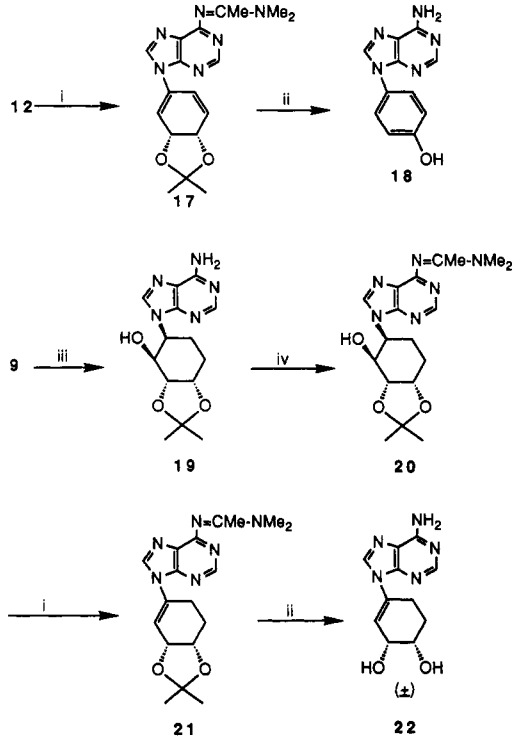
Scheme IV[a]



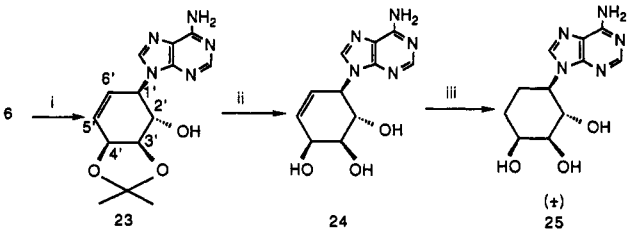
[a] Reagents: (i) DAST, DMAP, CH_2Cl_2, −78 °C to rt; (ii) (a) aqueous NH_4OH, (b) aqueous HCl, (c) Dowex-50W (H^+); (iii) PtO_2/H_2, 144 h; (iv) *N,N*-dimethylacetamide dimethyl acetal, MeOH, DMSO.

transformations shown in Scheme III and IV were carried out. Deprotection of **9** yielded the (trihydroxycyclohexenyl)adenine **10**, which upon hydrogenation yielded the (trihydroxycyclohexanyl)adenine **11** (Scheme III).

Compound **9** was also converted to the deoxy derivatives **15** and **16** (Scheme III). These derivatives were prepared by treatment of **9** with *N,N*-dimethylacetamide dimethyl acetal in MeOH and DMSO[13] to yield **12** in 64% yield. Reaction of **12** with *n*-BuLi followed by addition of CS_2 and MeI afforded **13** in 90% yield. Treatment of **13** with Bu_3SnH in the presence of AIBN[14] in dioxane afforded **14** in 80% yield. Removal of the methylamidene protecting group in **14** with 30% aqueous NH_4OH, followed by dilute aqueous HCl afforded **15** in 68% yield. Hydrogenation of **15** in the presence of Pd–C afforded **16** in 93% yield.

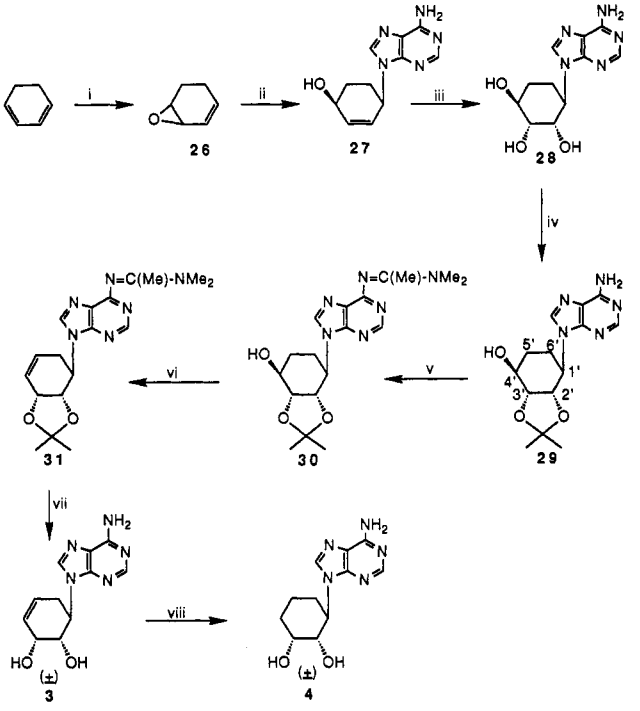
Attempts were also made to prepare the 2′-fluoro analogs of **15** and **16**. Treatment of **12** with (diethylamino)sulfur trifluoride (DAST) in the presence of DMAP in CH_2Cl_2[15] unexpectedly yielded the diene **17** in 90% yield (Scheme IV). The assignment of the structure for **17** was supported by the ^{1}H-NMR spectrum, which showed three olefinic protons appearing as two doublets at δ 6.57 (J = 10 Hz) and δ 6.31 (J = 3.5 Hz) and a doublet of doublets at δ 6.19 (J = 10 Hz, J = 3.5 Hz). Treatment of **17** with 30% aqueous NH_4OH and dilute aqueous HCl afforded the N^9-substituted adenine **18** in 71% yield (Scheme IV). Spectral characterization revealed that the adenine moiety in **18** is para to the hydroxyl group, providing strong evidence that 1,2-addition to yield **9**, not 1,4-addition to yield

Scheme V[a]



[a] Reagents: (i) adenine, K_2CO_3, DMAC, 130 °C, 2 h; (ii) aqueous HCl, (b) Dowex-50W (H^+); (iii) Pd–C/H_2.

Scheme VI



[a] Reagents: (i) *m*-CPBA, CH_2Cl_2, 0 °C; (ii) $[(i\text{-}C_3H_7O)_3P]_4Pd$, adenine, THF, DMSO; (iii) OsO_4, NMO, acetone; (iv) DMP, $HClO_4$, acetone; (v) *N,N*-dimethylacetamide dimethyl acetal, dioxane; (vi) DAST, CH_2Cl_2; (vii) (a) aqueous NH_4OH; (b) aqueous HCl; (c) Dowex-50W (H^+); (viii) Pd–C/H_2.

5, was observed in the adenine reaction with **6** (Scheme III).[16]

In another attempt to prepare a fluorine derivative, compound **9** was catalytically reduced (PtO_2) to yield **19** in 90% yield (Scheme IV). Treatment of **19** with *N,N*-dimethylacetamide dimethyl acetal in MeOH and DMSO afforded the methylamidene-protected compound **20** in 61% yield. Treatment of **20** with DAST again afforded elimination product **21** in 95% yield. Deprotection of **21** afforded the (dihydroxycyclohexanyl)adenine **22** in 67% yield.

In an effort to determine the role of the Pd(0) catalyst in the reaction of the monoepoxide **6** with adenine, the reaction was run in the absence of catalyst. As expected, the reaction of the monoepoxide **6** with adenine and K_2CO_3

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(16) The 1D NOE spectra were obtained with 50 mM solutions (DMSO, not degassed). The nonspinning samples (27 °C) were preirradiated for 5 s, followed by acquisition for 2.7 s (90° pulse). Blanks were run at the beginning and end of the list of frequencies. Each frequency used 16 real scans and 4 dummy scans, repeating this process 4 times. The spectra were transformed and subtracted and the percent enhancement calculated, correcting the intensities for percent saturation of the irradiated peak.

in DMAC afforded **23** in 50% yield (Scheme V), the result of trans opening of the epoxide. Deprotection of **23** yielded the (trihydroxycyclohexenyl)adenine **24** in 87% yield, which was reduced with Pd–C/H_2 to afford the (trihydroxycyclohexanyl)adenine **25** in 98% yield (Scheme V).

Since the Pd(0)-catalyzed nucleophilic addition of adenine to the allylic epoxide **6** failed to yield **5**, an alternative pathway to the desired adenine analogs **3** and **4** starting from 1,3-cyclohexadiene was devised (Scheme VI). Epoxidation of 1,3-cyclohexadiene, by a modification of a previously reported procedure using *m*-CPBA,[17] afforded 3,4-epoxycyclohexene (**26**) in 85% yield. Reaction of adenine and **26** with the Pd(0) catalyst, run under the same conditions as described for **9**, afforded in 35% yield the cis-1,4-alkylated product **27**. Apparently, the presence of the protected diol in **6** effected the cis-1,2-addition, although the reason for this is unclear at present. Upon treatment of **27** with catalytic amounts of OsO_4 and *N*-methylmorpholine *N*-oxide (NMO)[11,18] in aqueous acetone (2,3,4-trihydroxycyclohexanyl)adenine **28** was isolated in 95% yield. This result is consistent with the cis-hydroxylation of **27** proceeding from the sterically least-hindered face, which is trans to both the 4′-hydroxy group and the 1′-adenine moiety. Treatment of **28** in dimethoxypropane afforded **29** in 85% yield. A NOE study of **29** showed that irradiation of the signal for the 1′-proton enhanced only the signal for the cis 6′-proton (see the supplementary material). This result confirms the assignment of a trans relationship between the adenine moiety at C-1′ and the oxygen at C-2′. Irradiation of the signal for the 4′-proton showed significant NOE only on the signal for the 5′-protons. These results suggest a trans relationship between the 3′- and 4′-protons.

As shown in Scheme VI, compound **29** served as a convenient synthetic precursor to the desired (dihydroxycyclohexenyl)adenine **3** and the (dihydroxycyclohexanyl)adenine **4**. Protection of the 6-NH_2 group of **29** as the methylamidene derivative afforded **30** in 88% yield, which upon treatment with DAST in CH_2Cl_2 yielded the protected dihydroxycyclohexenyl **31** in 90% yield. In our hands, DAST was utilized in several cases to afford unexpected dehydration products in high yields. Deprotection of **31** by treatment with aqueous NH_4OH followed by HCl afforded **3** in 70% yield. Catalytic reduction of **3** afforded **4** in 95% yield.

Inhibition of AdoHcy Hydrolase. Using methodology previously described by our laboratory,[3b,19] the hydroxylated cyclohexenyladenines (**3**, **10**, **15**, **22**, and **24**) and cyclohexanyladenines (**4**, **11**, **16**, **25**, and **28**) were evaluated as potential inhibitors of purified bovine liver AdoHcy hydrolase. Compound **1**, a potent inhibitor of AdoHcy hydrolase,[3b] was used as a reference compound for these studies. Under the enzyme assay conditions described in the Experimental Section, compound **1** at concentrations of 1 and 10 μM produced 95.4 and 97.7% inhibition, respectively. When the cyclohexenyladenines (**3**, **10**, **15**, **22**, and **24**) and cyclohexanyladenines (**4**, **11**, **16**, **25**, and **28**) were treated in this assay system, only analog **3** produced more than 20% inhibition of AdoHcy hydrolase activity (1 μM, 26.7%; 10 μM, 59.6%). The relative inactivity of these cyclohexenyl- and cyclohexanyladenines, including compound **3**, did not warrant further biological evaluation.

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Experimental Section

Melting points are uncorrected. Elemental analyses were performed in the Department of Medicinal Chemistry, University of Kansas. Column chromatography was accomplished with 70–230-mesh silica gel (Aldrich Chemical Co., Milwaukee, WI) unless otherwise stated. Ion-exchange chromatography was carried out with Dowex-50W (H^+), dry mesh 100–200, 4% cross-linked (Sigma Chemical Co., St. Louis, MO). All reactions were run under argon atmosphere except where H_2O was used as solvent.

***cis*-1,2-(Isopropylidenedioxy)cyclohexa-3,5-diene (7).** To a −10 to −15 °C solution of *cis*-3,5-cyclohexadiene-1,2-diol (5 g, 44.6 mmol) and 20 mL of 2,2-dimethoxypropane in CH_2Cl_2 (50 mL) was added a catalytic amount (20 mg) of *p*-toluenesulfonic acid, and the reaction mixture was stirred at this temperature for 1 h. After 20 mL of 10% aqueous NaOH was added, the reaction mixture was stirred for 5 min. The organic layer was separated, washed with H_2O (4 × 50 mL) and dried (Na_2SO_4), and the solvent was evaporated to give **7** (6.63 g, 98%) as a colorless oil: IR (neat) 3150, 2990, 2940, 2890, 1450, 1420, 1370, 1250, 1210, 1160, 1030, 875 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.95 (m, 2 H), 5.84 (m, 2 H), 4.61 (s, 2 H), 1.38 (s, 3 H), 1.36 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 125.0, 123.1, 104.3, 70.5, 26.5, 24.6.

(1*S*,2*S*,5*S*,6*S*)-1,2-(Isopropylidenedioxy)-5,6-epoxy-3-cyclohexene (6). To a stirred 0 °C solution of **7** (4 g, 26.4 mmol) in CH_2Cl_2 (50 mL) was added *m*-chloroperbenzoic acid (55%, 9.38 g, 30 mmol). After the mixture was stirred at 0 °C for 8 h, the precipitated *m*-chlorobenzoic acid was removed by filtration. The filtrate was cooled to −78 °C and again filtered, and the filtrate was concentrated. The residue was passed through a short column of Florisil (100–200 mesh, Fisher Scientific) using CH_2Cl_2 and hexane (1:3), affording 3.6 g (81%) of **6** as a colorless oil: IR (neat) 2930, 2900, 1380, 1370, 1240, 1070, 1050, 825 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.06 (ddd, 1 H, J_1 = 8, J_2 = 5, J_3 = 2), 5.79 (ddd, 1 H, J_1 = 8, J_2 = 2, J_3 = 2), 4.78 (dd, 1 H, J_1 = 7, J_2 = 2), 4.46 (dt, 1 H, J_1 = 7, J_2 = 4), 3.55 (dd, 1 H, J_1 = 3.5, J_2 = 2), 3.35 (ddd, 1 H, J_1 = 7, J_2 = 3.5, J_3 = 2), 1.41 (s, 6 H); ^{13}C NMR ($CDCl_3$) δ 132.4, 123.9, 110.9, 71.2, 71.1, 49.6, 46.8, 28.2, 26.3.

(1*R*,2*R*,3*R*,6*S*)-6-Methoxy-4-cyclohexene-1,2,3-triol (8). Method A. To a stirred solution of monoepoxide **6** (168 mg, 1 mmol) in $CHCl_3$ (5 mL) and MeOH (10 mL), was added portionwise *d*-10-camphorsulfonic acid (30 mg, 0.12 mmol). After 16 h the reaction mixture was concentrated, and the residue was dissolved in $CHCl_3$ (20 mL), poured into water (10 mL), and stirred for 30 min. The organic layer was separated, dried (Na_2SO_4), and concentrated. The residue was flash chromatographed ($CHCl_3$–MeOH, 19:1) to yield 140 mg (87%) of pure **8** as an oil: IR (neat) 3400, 2920, 2820, 1640, 1400, 1190, 1070, 985, 940, 860, 795 cm^{-1}; ^1H NMR (DMSO-d_6) δ 5.84 (m, 2 H), 4.82 (br s, 3 H, exchanged with D_2O), 4.21 (m, 1 H), 3.65 (m, 2 H), 3.49 (s, 3 H), 3.43 (m, 1 H); ^{13}C NMR (DMSO-d_6) δ 129.4, 128.9, 82.0, 71.5, 70.6, 66.4, 56.8; MS (CI, NH_3 in MeOH) *m*/*z* 161 (M^+ + H), 143, 129, 125, 113, 100, 71. Anal. Calcd for $C_7H_{12}O_4$: C, 52.49; H, 7.55. Found: C, 52.53; H, 7.58.

Method B. A solution of 6-methoxy-4-cyclohexene-1,2,3-triol dibenzoate[11] (370 mg, 1 mmol) in Et_3N/MeOH/H_2O (1:5:1, 10 mL) was stirred at room temperature for 12 h. The reaction mixture was concentrated, and the residue was flash chromatographed ($CHCl_3$–MeOH, 19:1) to yield 128 mg (80%) of **8**. The spectral characteristics of **8** were identical with those of the product obtained by method A.

9-[(1′*S*,2′*S*,3′*R*,4′*S*)-2′-Hydroxy-3′,4′-(isopropylidenedioxy)-5′-cyclohexenyl]adenine (9). To a stirred mixture of $Pd(OAc)_2$ (170 mg, 0.75 mmol) in dry THF (5 mL) at 0 °C was added triisopropyl phosphite (1.85 mL, 7.5 mmol) followed by dropwise addition of *n*-BuLi (0.97 mL, 1.6 M solution in hexane, 1.5 mmol). The clear solution containing $[(i\text{-}C_3H_7O)_3P]_4Pd$ was added dropwise to a mixture of adenine (1.35 g, 10 mmol) and monoepoxide **6** (1.68 g, 10 mmol) in THF–DMSO (1:1 40 mL) at 0 °C. After the reaction mixture was stirred at 0 °C for 3 h, the temperature was increased to room temperature and the mixture was stirred for an additional 16 h. The reaction was quenched with saturated aqueous NH_4Cl solution, and the precipitate was filtered and washed thoroughly with CH_2Cl_2 (4 × 50 mL) and H_2O (4 × 50 mL). The precipitate was dried under vacuum to yield **9** (2.77 g, 90%) as a white solid: mp >300 °C; IR (KBr) 3300,

Table I. Nuclear Overhauser Enhancement Experimental Data for 9

preirradiated peak (δ)	percent enhancement						
	δ 5.94 (C_5-H)	5.80 (C_6-H)	5.64 (C_2-OH)	5.31 (C_1-H)	4.67 (C_4-H)	4.31 (C_3-H)	4.14 (C_2-H)
5.94					1.0		
5.80				1.4			
5.64					1.1	2.3	4.0
5.31		1.8					4.5
4.67	1.4		1.0			3.4	
4.31			2.5		3.5		
4.14			4.3	3.8			

3140, 2990, 1690, 1610, 1230, 1100, 1045 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 8.14 (s, 1 H), 7.90 (s, 1 H), 7.24 (s, 2 H, exchanged with D_2O), 5.94 (d, 1 H, J = 10), 5.80 (d, 1 H, J = 10), 5.64 (s, 1 H, exchanged with D_2O), 5.31 (br s, 1 H), 4.67 (t, 1 H, J = 2.5), 4.31 (t, 1 H, J = 4.7), 4.14 (m, 1 H), 1.36 (s, 3 H), 1.33 (s, 3 H); ^{13}C NMR (DMSO-d_6) δ 155.9, 152.2, 149.3, 140.5, 129.6, 124.9, 118.4, 108.5, 75.4, 70.7, 67.4, 50.4, 27.9, 26.4; MS (EI) 303 (M^+), 288, 245, 228, 216, 186, 177, 135. Anal. Calcd for $C_{14}H_{17}N_5O_3$: C, 55.44; H, 5.65; N, 23.09. Found: C, 55.35; H, 5.61; N, 22.95.

9-[(1′*S*,2′*S*,3′*S*,4′*S*)-2′,3′,4′-Trihydroxy-5′-cyclohexenyl]-adenine (10). A mixture of compound **9** (200 mg, 0.66 mmol) and 10% aqueous HCl (20 mL) was stirred at room temperature for 2 h. The reaction mixture was concentrated in vacuo and the residue passed through a Dowex-50W (H^+) resin column (elution with 10% aqueous NH_4OH), affording 156 mg (90%) of **10** as a white solid: mp 263–264 °C; IR (KBr) 3340, 3120, 1670, 1600, 1420, 1335, 1300, 1120, 1065, 1025 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 8.20 (s, 1 H), 7.90 (s, 1 H), 7.25 (s, 2 H, exchanged with D_2O), 5.88 (d, 1 H, J = 11), 5.65 (dd, 1 H, J_1 = 11, J_2 = 1.5), 5.46 (br s, 1 H), 5.35 (d, 1 H, J = 5, exchanged with D_2O), 5.14 (d, 1 H, J = 3.5, exchanged with D_2O), 4.79 (d, 1 H, J = 7.7, exchanged with D_2O), 4.37 (br s, 1 H), 4.05 (m, 1 H), 3.89 (br s, 1 H); ^{13}C NMR (DMSO-d_6) δ 156.2, 152.4, 149.6, 140.9, 133.9, 123.8, 118.7, 71.4, 69.6, 64.8, 51.2; MS (EI) m/z 263 (M^+), 246, 228, 204, 174, 136, 135. Anal. Calcd for $C_{11}H_{13}N_5O_3$: C, 50.19; H, 4.98; N, 26.60. Found: C, 50.23; H, 4.91; N, 26.52.

9-[(1′*S*,2′*S*,3′*S*,4′*S*)-2′,3′,4′-Trihydroxycyclohexanyl]-adenine (11). A suspension of **10** (236 mg, 1 mmol) and 10% Pd–C (20 mg) in CH_3OH (50 mL) was hydrogenated at 50 psi in a Parr apparatus for 48 h. The reaction mixture was filtered, and the filtrate was concentrated to give 236 mg (99%) of **11**: mp 267–269 °C dec; IR (KBr) 3340, 3140, 1670, 1600, 1480, 1420, 1340, 1300, 1100, 1070, 1010 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 8.10 (s, 2 H), 7.15 (s, 2 H, exchanged with D_2O), 5.35 (d, 1 H, J = 4.5, exchanged with D_2O), 4.89 (d, 1 H, J = 3, exchanged with D_2O), 4.78 (m, 1 H), 4.41 (d, 1 H, J = 6.2, exchanged with D_2O), 3.78 (m, 3 H), 2.20 (m, 1 H), 1.75 (m, 3 H); ^{13}C NMR (DMSO-d_6) δ 155.9, 152.0, 149.1, 140.1, 118.4, 72.7, 71.1, 51.2, 27.4, 24.1; MS (EI) m/z 265 (M^+), 248, 203, 190, 177, 136. Anal. Calcd for $C_{11}H_{15}N_5O_3$: C, 49.81; H, 5.70; N, 26.40. Found: C, 49.85; H, 5.65; N, 26.47.

9-[(1′*S*,2′*S*,3′*R*,4′*S*)-2′-Hydroxy-3′,4′-(isopropylidenedioxy)-5′-cyclohexenyl]-*N*^{6}-[1-(dimethylamino)ethylidene]-adenine (12). To a stirred solution of **9** (1 g, 3.3 mmol) in MeOH–DMSO (1:1, 50 mL) was added dropwise *N*,*N*-dimethylacetamide dimethyl acetal (1.5 mL, 9 mmol), and the contents were stirred at 90 °C for 16 h. The reaction mixture was concentrated, and the residue was chromatographed (CH_2Cl_2–MeOH, 9:1) to afford 0.8 g (64%) of **12** as a white powder: mp 187–189 °C; IR (KBr) 3200, 2980, 2970, 2940, 1560, 1550, 1395, 1330, 1220, 1160, 1040 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 8.42 (s, 1 H), 7.89 (s, 1 H), 5.97 (d, 1 H, J = 10), 5.84 (d, 1 H, J = 10), 5.73 (br s, 1 H, exchanged with D_2O), 5.39 (m, 1 H), 4.69 (m, 1 H), 4.35 (m, 1 H), 4.20 (m, 1 H), 3.10 (s, 6 H), 2.05 (s, 3 H), 1.38 (s, 3 H), 1.35 (s, 3 H); ^{13}C NMR (DMSO-d_6) δ 160.7, 159.8, 151.9, 150.8, 142.2, 129.7, 124.9, 124.7, 108.5, 75.4, 70.7, 67.3, 50.5, 38.5, 38.2, 27.9, 26.4, 17.0; MS (EI) m/z 373 (M^+), 357, 314, 302, 285, 205, 134. Anal. Calcd for $C_{18}H_{24}N_6O_3$: C, 58.05; H, 6.49; N, 22.56. Found: C, 59.1; H, 6.43; N, 22.49.

9-[(1′*S*,2′*S*,3′*S*,4′*S*)-2′-[(Methylthio)(thiocarbonyl)]-oxy]-3′,4′-(isopropylidenedioxy)-5′-cyclohexenyl]-*N*^{6}-[1-(dimethylamino)ethylidene]adenine (13). To a cooled (−15 °C) and stirred solution of **12** (950 mg, 2.5 mmol) in THF (50 mL) was added dropwise *n*-BuLi (2 mL, 1.6 M solution in hexane, 3.1 mmol). After 10 min, CS_2 (3 mL) was added dropwise, followed (after 30 min) by MeI (2 mL). Stirring was continued at −15 °C for 30 min. The solvents were evaporated under reduced pressure, while maintaining the temperature below 40 °C. The residue was taken up in CH_2Cl_2 (50 mL), washed with saturated aqueous NH_4Cl solution (50 mL) followed by H_2O (2 × 50 mL), dried (Na_2SO_4), and concentrated. The crude product was purified by chromatography (CH_2Cl_2–MeOH, 97:3), yielding 1.08 g (90%) of **13** as a white solid: mp 216–218 °C: IR (KBr) 2980, 2960, 1565, 1545, 1395, 1190, 1060, 1040 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.62 (s, 1 H), 7.82 (s, 1 H), 6.44 (t, 1 H, J = 4.5), 6.24 (dt, 1 H, J_1 = 10, J_2 = 2.5), 5.94 (dd, 1 H, J_1 = 10, J_2 = 2.5), 5.87 (d, 1 H, J = 2.5), 4.84 (m, 1 H), 4.62 (m, 1 H), 3.19 (br d, 6 H), 2.42 (s, 3 H), 2.14 (s, 3 H), 1.52 (s, 3 H), 1.44 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 215.2, 161.1, 160.1, 153.1, 151.2, 140.8, 131.0, 125.5, 124.3, 110.4, 77.9, 72.0, 71.3, 49.0, 38.4, 38.1, 27.8, 26.3, 19.3, 17.3; MS (EI) m/z 462 (M^+), 447, 415, 354, 339, 297, 284, 252, 205, 149. Anal. Calcd for $C_{20}H_{26}N_6O_3S_2$: C, 51.93; H, 5.66; N, 18.17. Found: C, 51.98; H, 5.59; N, 18.12.

9-[(1′*R*,3′*R*,4′*S*)-3′,4′-(Isopropylidenedioxy)-5′-cyclohexenyl]-*N*^{6}-[1-(dimethylamino)ethylidene]adenine (14). A mixture of **13** (500 mg, 1.1 mmol), Bu_3SnH (1 mL) and α,α′-azoisobutyronitrile (AIBN, 25 mg) in dry dioxane (25 mL) was stirred and refluxed for 4 h. The reaction mixture was concentrated under reduced pressure, and the residue was flash chromatographed over silica gel (CH_2Cl_2–MeOH, 97:3) to give 305 mg (80%) of **14** as a white powder: mp 140–142 °C; IR (KBr) 2980, 2920, 1620, 1570, 1550, 1400, 1335, 1320, 1225, 860 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.63 (s, 1 H), 7.89 (s, 1 H), 5.98 (m, 2 H), 5.45 (m, 1 H), 4.70 (m, 1 H), 4.57 (m, 1 H), 3.23 (s, 3 H), 3.18 (s, 3 H), 2.71 (m, 1 H), 2.35 (m, 1 H), 2.19 (s, 3 H), 1.48 (s, 3 H), 1.43 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 160.9, 160.3, 152.8, 150.8, 140.1, 130.0, 128.7, 126.1, 108.9, 71.9, 70.9, 47.4, 32.8, 32.4, 27.7, 26.3, 17.2, 13.4; MS (EI) m/z 356 (M^+), 341, 298, 286, 205, 134, 120. Anal. Calcd for $C_{18}H_{24}N_6O_2$: C, 60.66; H, 6.79; N, 23.58. Found: C, 60.59; H, 6.76; N, 23.54.

9-[(1′*R*,3′*R*,4′*S*)-3′,4′-Dihydroxy-5′-cyclohexenyl]adenine (15). To a solution of **14** (300 mg, 0.84 mmol) in CH_3OH (5 mL) was added 30% aqueous NH_4OH (5 mL) and the reaction mixture was stirred for 12 h. The reaction mixture was concentrated, and the residue was dissolved in 10% aqueous HCl (15 mL) and stirred for 3 h. After evaporation of the solvent, the residue was purified through Florisil (CH_2Cl_2–MeOH, 9:1) to yield 146 mg (68%) of **15** as a white solid: mp 279–280 °C; IR (KBr) 3370, 3330, 3160, 2960, 1660, 1600, 1570, 1420, 1280, 1200, 1185, 1060 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 8.15 (s, 1 H), 8.09 (s, 1 H), 7.26 (s, 2 H, exchanged with D_2O), 5.82 (d, 1 H, J = 10), 5.72 (d, 1 H, J = 10), 5.32 (m, 1 H), 4.92 (d, 1 H, J = 6.4, exchanged with D_2O), 4.76 (d, 1 H, J = 3, exchanged with D_2O), 4.19 (m, 1 H), 3.98 (br d, 1 H), 2.25 (m, 1 H), 2.10 (m, 1 H); ^{13}C NMR (DMSO-d_6) δ 155.9, 152.3, 149.1, 139.4, 133.4, 126.3, 118.9, 66.7, 66.2, 48.3, 34.4; MS (EI) m/z 247 (M^+), 230, 204, 186, 173, 136. Anal. Calcd for $C_{11}H_{13}N_5O_2$: C, 53.44; H, 5.30; N, 28.32. Found: C, 53.38; H, 5.27; N, 28.25.

9-[(1′*S*,3′*R*,4′*S*)-3′,4′-Dihydroxycyclohexanyl]adenine (16). Compound **15** (247 mg, 1 mmol) was hydrogenated as described for preparation of **11**, yielding 230 mg (93%) of **16** as a white solid: mp 223–225 °C; IR (KBr) 3380, 3340, 3150, 1660, 1600, 1570, 1480, 1410, 1330, 1300, 1250, 1070 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 8.22 (s, 1 H), 8.14 (s, 1 H), 7.25 (s, 2 H, exchanged with D_2O), 4.74 (m, 2 H, exchanged with D_2O), 3.94 (br s, 1 H), 3.56 (m, 2 H), 2.20 (m, 1 H), 1.90 (m, 4 H), 1.65 (m, 1 H); ^{13}C NMR (DMSO-d_6) δ 155.9, 152.1, 149.1, 139.2, 118.9, 69.9, 68.4, 48.3, 36.9, 30.2, 27.3; MS (EI) m/z 249 (M^+), 232, 162, 136. Anal. Calcd for $C_{11}H_{15}N_5O_2$: C, 53.00; H, 6.07; N, 28.09. Found: C, 53.21; H, 6.11; N, 28.02.

9-[(3′*R*,4′*S*)-3′,4′-(Isopropylidenedioxy)-1′,5′-cyclo-

hexadienyl]-N^6-[1-(dimethylamino)ethylidene]adenine (17). To a stirred solution of **12** (200 mg, 0.536 mmol) and DMAP (200 mg) in CH_2Cl_2 (30 mL) at −78 °C was added dropwise DAST (0.4 mL, 2.5 mmol). The reaction mixture was allowed to warm to room temperature over 2 h, and then was stirred at room temperature for 22 h. The reaction was quenched with saturated aqueous $NaHCO_3$. The organic layer was separated, washed with H_2O (2 × 50 mL), dried (Na_2SO_4), and concentrated. The residue was purified through silica gel (CH_2Cl_2–MeOH, 97:3) to obtain 180 mg (90%) of **17** as an amorphous powder: mp 160–162 °C; IR (KBr), 2980, 2920, 1600, 1565, 1400, 1395, 1230, 1055, 1040, 1020 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.62 (s, 1 H), 8.05 (s, 1 H), 6.57 (d, 1 H, J = 10), 6.31 (d, 1 H, J = 3.5), 6.19 (dd, 1 H, J_1 = 10, J_2 = 3.5), 4.93 (dd, 1 H, J_1 = 9, J_2 = 3.5), 4.85 (dd, 1 H, J_1 = 9, J_2 = 3.5), 3.20 (br d, 6 H), 2.18 (s, 3 H), 1.47 (s, 3 H), 1.44 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 161.2, 166.4, 153.3, 150.7, 139.2, 131.3, 129.2, 126.1, 121.6, 115.5, 105.2, 69.9, 69.7, 38.3, 38.2, 26.5, 24.4, 17.2; MS (EI) m/z 354 (M^+), 339, 298, 284. Anal. Calcd for $C_{18}H_{22}N_6O_2$: C, 61.00; H, 6.26; N, 23.71. Found: C, 61.12; H, 6.30; N, 23.64.

9-(*p*-Hydroxyphenyl)adenine (18). Compound **17** (177 mg, 0.5 mmol) was treated with 30% aqueous NH_4OH, followed by 10% aqueous HCl as described for the preparation of **15**, to afford 80 mg (71%) of **18** as a light brown solid: mp >300 °C; IR (KBr) 3260, 3230, 3080, 1675, 1595, 1520, 1300, 1235 cm^{-1}; ^1H NMR (DMSO-d_6) δ 9.82 (br s, 1 H, exchanged with D_2O), 8.42 (s, 1 H), 8.18 (s, 1 H), 7.59 (br d, 2 H), 7.35 (s, 2 H, exchanged with D_2O), 6.94 (br d, 2 H); ^{13}C NMR (DMSO-d_6) δ 184.4, 172.6, 156.7, 156.2, 152.9, 149.3, 139.8, 126.2, 124.8, 118.9, 115.7; MS (EI) m/z 227 (M^+), 200, 149. Anal. Calcd for $C_{11}H_9N_5O$: C, 58.15; H, 3.99; N, 30.72. Found: C, 58.19; H, 3.96; N, 30.65.

9-[(1′*S*,2′*S*,3′*R*,4′*S*)-2′-Hydroxy-3′,4′-(isopropylidenedioxy)cyclohexanyl]adenine (19). A suspension of **9** (1 g, 3.3 mmol) and PtO_2 (25 mg) in CH_3OH (150 mL) was hydrogenated at 60 psi for 6 d. The reaction mixture was filtered and the filtrate concentrated to yield 900 mg (90%) of **19** as a white powder: mp 180–182 °C; IR (KBr) 3320, 3260, 3180, 2980, 1660, 1610, 1050 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.13 (s, 2 H), 7.20 (s, 2 H, exchanged with D_2O), 5.75 (br s, 1 H, exchanged with D_2O), 4.80 (br d, 1 H), 4.31 (m, 1 H), 4.15 (m, 1 H), 3.99 (br s, 1 H), 2.15 (m, 2 H), 1.78 (m, 2 H), 1.51 (s, 3 H), 1.29 (s, 3 H); ^{13}C NMR (DMSO-d_6) δ 155.8, 152.1, 149.5, 139.9, 119.2, 107.9, 77.5, 71.3, 67.4, 51.1, 27.9, 26.6, 25.6, 21.6; MS (EI) m/z 305 (M^+), 290, 247, 190, 162, 152, 136. Anal. Calcd for $C_{14}H_{19}N_5O_3$: C, 55.07; H, 6.27; N, 22.94. Found: C, 55.01; H, 6.30; N, 22.99.

9-[(1′*S*,2′*S*,3′*R*,4′*S*)-2′-Hydroxy-3′,4′-(isopropylidenedioxy)cyclohexenyl]-N^6-[1-(dimethylamino)ethylidene]adenine (20). The 6-NH_2 group in compound **19** (500 mg, 1.15 mmol) was protected as the methylamidine using the procedure described for preparation of **12**, yielding 390 mg (61%) of **20** (amorphous powder): mp 172–174 °C; IR (KBr) 3170, 2980, 2920, 2880, 1580, 1550, 1390 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.47 (s, 1 H), 7.99 (s, 1 H), 7.1 (br s, 1 H, exchanged with D_2O), 4.81 (m, 1 H), 4.45 (br s, 1 H), 4.33 (m, 2 H), 3.15 (br d, 6 H), 2.28 (m, 2 H), 2.12 (s, 3 H), 1.89 (m, 2 H), 1.58 (s, 3 H), 1.38 (s, 3 H); ^{13}C NMR (DMSO-d_6) δ 161.7, 160.0, 151.8, 149.9, 141.9, 125.8, 108.7, 80.9, 76.6, 72.1, 69.2, 38.5, 38.3, 27.8, 25.1, 24.9, 22.4, 17.4; MS (EI) m/z 374 (M^+), 359, 330, 316, 304, 259, 246, 231, 205, 134, 120. Anal. Calcd for $C_{18}H_{26}N_6O_3$: C, 57.74; H, 7.00; N, 22.44. Found: C, 57.80; H, 6.97; N, 22.35.

9-[(3′*R*,4′*S*)-3′,4′-(Isopropylidenedioxy)-1-cyclohexenyl]-N^6-[1-(dimethylamino)ethylidene]adenine (21). Compound **20** (250 mg, 0.535 mmol) was converted to **21** (225 mg, 95%) as described for preparation of **17**: mp 110–112 °C; IR (KBr) 2980, 2920, 1600, 1565, 1400, 1330, 1240, 1210, 1050 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.61 (s, 1 H), 7.91 (s, 1 H), 6.31 (s, 1 H), 4.82 (m, 1 H), 4.45 (m, 1 H), 3.18 (br d, 6 H), 2.81 (m, 2 H), 2.21 (m, 2 H), 2.15 (s, 3 H), 1.45 (s, 3 H), 1.42 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 161.2, 160.6, 153.1, 150.9, 139.4, 135.6, 126.3, 118.4, 108.9, 71.8, 71.7, 38.3, 38.1, 28.1, 26.3, 25.1, 22.5, 17.3; MS (EI) m/z 356 (M^+), 341, 300, 286, 242, 212, 158. Anal. Calcd for $C_{18}H_{24}N_6O_2$: C, 60.66; H, 6.79; N, 23.58. Found: C, 60.58; H, 6.75; N, 23.61.

9-[(3′*R*,4′*S*)-3′,4′-Dihydroxy-1′-cyclohexenyl]adenine (22). Compound **21** (118 mg, 0.33 mmol) was deprotected as described for preparation of **15** to yield 56 mg (67%) of **22** as a white powder: mp 150–152 °C; ^1H NMR (DMSO-d_6) δ 8.25 (s, 1 H), 8.15 (s, 1 H), 7.30 (s, 2 H, exchanged with D_2O), 6.39 (br s, 1 H), 4.89 (br s, 1 H, exchanged with D_2O), 4.59 (br s, 1 H, exchanged with D_2O), 4.19 (m, 1 H), 3.75 (m, 1 H), 2.79 (m, 1 H), 2.59 (m, 1 H), 1.92 (m, 1 H), 1.72 (m, 1 H); ^{13}C NMR (DMSO-d_6) δ 156.2, 152.7, 149.2, 139.0, 133.9, 120.2, 119.4, 66.8, 65.8, 25.6, 24.5. Anal. Calcd for $C_{11}H_{13}N_5O$: C, 53.44; H, 5.30; N, 28.32. Found: C, 53.50; H, 5.27; N, 28.39.

9-[(1′*R*,2′*S*,3′*R*,4′*S*)-2′-Hydroxy-3′,4′-(isopropylidenedioxy)-5′-cyclohexenyl]adenine (23). A mixture of monoepoxide **6** (504 mg, 3 mmol), adenine (405 mg, 3 mmol), and K_2CO_3 (200 mg) in *N*,*N*-dimethylacetamide (DMAC) (20 mL) was stirred and heated at 130 °C for 2 h. The reaction mixture was concentrated, passed through silica gel (CH_2Cl_2–MeOH, 19:1), and recrystallized from MeOH to give 405 mg (50%) of **23**: mp 265–266 °C; IR (KBr) 3360, 3320, 3180, 1655, 1600, 1580, 1480, 1330, 1300, 1245, 1210, 1070, 720 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.10 (s, 1 H), 8.07 (s, 1 H), 7.19 (s, 2 H, exchanged with D_2O), 5.98 (dt, 1 H, J_1 = 10, J_2 = 3), 5.86 (dd, 1 H, J_1 = 10, J_2 = 1), 5.39 (d, 1 H, J = 6, exchanged with D_2O), 4.90 (dd, 1 H, J_1 = 9, J_2 = 2), 4.75 (m, 1 H), 4.16 (m, 1 H), 3.99 (m, 1 H), 1.42 (s, 3 H), 1.33 (s, 3 H); ^{13}C NMR (DMSO-d_6) δ 155.9, 152.2, 149.9, 140.5, 130.6, 125.5, 119.1, 108.9, 78.1, 72.2, 70.0, 56.6, 27.9, 25.6; MS (EI) m/z 303 (M^+), 288, 244, 228, 216, 200, 136. Anal. Calcd for $C_{14}H_{17}N_5O_3$: C, 55.44; H, 5.65; N, 23.09. Found: C, 55.35; H, 5.59; N, 23.11.

9-[(1′*R*,2′*S*,3′*S*,4′*S*)-2′,3′,4′-Trihydroxy-5′-cyclohexenyl]adenine (24). Compound **23** (200 mg, 0.66 mmol) was deprotected as described earlier for preparation of **10**. Recrystallization from MeOH afforded 150 mg (87%) of **24**: mp 262–264 °C; IR (KBr) 3390, 3340, 3160, 1660, 1600, 1570, 1470, 1410, 1340, 1280, 1200, 1180, 1060 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.12 (s, 1 H), 8.10 (s, 1 H), 7.20 (s, 2 H, exchanged with D_2O), 5.91 (m, 1 H), 5.60 (m, 1 H), 5.21 (br s, 1 H, exchanged with D_2O), 4.99 (br s, 2 H, exchanged with D_2O), 4.89 (br s, 1 H), 4.16 (br s, 1 H), 4.10 (m, 1 H), 3.54 (m, 1 H); ^{13}C NMR (DMSO-d_6) δ 155.8, 152.1, 149.5, 140.0, 130.8, 127.1, 118.6, 71.6, 69.7, 65.9, 57.4; MS (EI) m/z 263 (M^+), 246, 204, 136. Anal. Calcd for $C_{11}H_{13}N_5O_3$: C, 50.19; H, 4.98; N, 26.60. Found: C, 50.22; H, 4.95; N, 26.51.

9-[(1′*R*,2′*S*,3′*S*,4′*S*)-2′,3′,4′-Trihydroxycyclohexanyl]adenine (25). Compound **24** (132 mg, 0.5 mmol) was hydrogenated as described above for preparation of **11**. Recrystallization from MeOH afforded 130 mg (98%) of **25**: mp 208–210 °C; IR (KBr) 3440, 3340, 3200, 1655, 1605, 1595, 1420, 1335, 1305, 1260, 1070 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.08 (s, 1 H), 8.05 (s, 1 H), 7.10 (s, 2 H, exchanged with D_2O), 4.80 (br d, 2 H, exchanged with D_2O), 4.59 (br s, 1 H, exchanged with D_2O) 4.15 (m, 2 H), 3.89 (br s, 1 H), 3.30 (br s, 1 H), 2.41 (m, 1 H), 1.65 (m, 3 H); ^{13}C NMR (DMSO-d_6) δ 155.9, 151.9, 149.7, 140.3, 119.1, 75.8, 70.6, 68.6, 58.8, 28.6, 25.2; MS (EI) m/z 265 (M^+), 248, 230, 190, 177, 136. Anal. Calcd for $C_{11}H_{15}N_5O_3$: C, 48.81; H, 5.70; N, 26.40. Found: C, 48.77; H, 5.74; N, 26.32.

3,4-Epoxycyclohexene (26). To a solution of cyclohexadiene (10 g, 0.125 M) in CH_2Cl_2 (50 mL) was added dropwise *m*-CPBA (22 g, 0.127 M) in CH_2Cl_2 (100 mL) at 0 °C. After the addition was complete, the mixture was stirred for an additional 3 h. The reaction mixture was filtered, and the filtrate was concentrated. The oily residue was distilled (bp 62–64 °C (65 mm)) to give 10 g (85%) of **26**[17] as an oil: IR (neat) 3030, 3010, 2980, 1630, 1420, 1020, 915, 855 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.94 (m, 2 H), 3.50 (m, 1 H), 3.23 (m, 1 H), 2.25 (m, 1 H), 2.05 (m, 2 H), 1.60 (m, 1 H); ^{13}C NMR ($CDCl_3$) δ 132.9, 123.1, 55.0, 46.9, 20.8, 20.6.

9-[(1′*R*,4′*S*)-4′-Hydroxy-2′-cyclohexenyl]adenine (27). To a cooled (0 °C) solution of $Pd(OAc)_2$ (170 mg, 0.75 mmol) in dry THF (10 mL) was added triisopropyl phosphite (1.85 mL, 7.5 mmol), followed by dropwise addition of *n*-BuLi (1.6 M solution in hexane, 0.97 mL, 1.5 mmol). The resulting solution containing $[(i\text{-}C_3H_7O)_3P]_4Pd$ was added dropwise to a mixture of **26** (960 mg, 10 mmol) and adenine (1.35 g, 10 mmol) dissolved in THF–DMSO (1:1, 50 mL) at 0 °C. After 3 h the reaction mixture was brought to room temperature and stirred for 24 h. The solvents were removed under reduced pressure, and the residue was passed through silica gel (CH_2Cl_2–MeOH; 8.5:1.5) and recrystallized from MeOH to afford 805 mg (35%) of **27** as a white solid: mp 176–177 °C; IR (KBr) 3320, 3180, 2940, 1650, 1635, 1595, 1570, 1460, 1410, 1300, 1205 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.17 (s, 1 H), 8.06 (s, 1 H), 7.32 (s, 2 H, exchanged with D_2O), 6.09 (d, 1 H, J_1 = 10), 5.83 (d, 1 H, J_1 = 10), 5.07 (br s, 1 H), 4.98 (s, 1 H, exchanged with

Table II. Nuclear Overhauser Enhancement Experimental Data for 29

preirradiated peak (δ)	percent enhancement							
	δ 5.23 (C_4-OH)	4.73 (C_2-H)	4.41 (C_1-H)	4.19 (C_3-H)	4.11 (C_4-H)	2.38 (C_6-H)	1.78 (C_5-H_2)	1.59 (C_6-H)
5.23				2.1	3.3		1.0	
4.73				3.9		1.9		
4.41								3.0
4.19	1.9	4.1						
4.11	3.2						5.2	
2.38		2.0					2.5	6.7
1.78	1.1		2.4		5.3	2.7		
1.59			3.1			6.5		

D_2O), 4.09 (br s, 1 H), 1.97 (m, 2 H), 1.81 (m, 1 H), 1.55 (m, 1 H); ^{13}C NMR (DMSO-d_6) δ 155.9, 152.3, 148.9, 139.6, 136.6, 125.6, 118.9, 63.1, 48.5, 27.9, 25.7; MS (EI) m/z 231 (M^+), 214, 186, 174, 162, 136. Anal. Calcd for $C_{11}H_{13}N_5O$: C, 57.13; H, 5.66; N, 30.28. Found: C, 57.20, H, 5.69; N, 30.35.

9-[(1′*R*,2′*R*,3′*S*,4′*S*)-2′,3′,4′-Trihydroxycyclohexanyl]-adenine (28). To a mixture of **27** (230 mg, 1 mmol) in 90% aqueous acetone (20 mL) was added 4-methylmorpholine *N*-oxide (NMO) (60 wt %, 0.2 mL) followed by OsO_4 (5 mg), and the contents were stirred for 3 d. The reaction mixture was filtered, and the solid precipitate was washed with acetone (2 × 50 mL) and H_2O (2 × 50 mL). The precipitate was recrystallized from MeOH and dried under vacuum, yielding **28** (252 mg, 95% yield): mp 290–292 °C; IR (KBr) 3320, 3230, 3160, 2960, 2930, 1690, 1610, 1570, 1420, 1300, 1215, 1085, 1060 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.10 (s, 2 H), 7.12 (s, 2 H, exchanged with D_2O), 4.93 (s 1 H, exchanged with D_2O), 4.87 (s, 1 H, exchanged with D_2O), 4.55 (s, 1 H, exchanged with D_2O), 4.48 (m, 1 H), 4.33 (m, 1 H), 3.81 (br d, 2 H), 2.30 (m, 2 H), 1.83 (m, 1 H), 1.59 (m, 1 H); ^{13}C NMR (DMSO-d_6) δ 156.2, 152.5, 150.3, 141.2, 119.4, 73.5, 69.5, 68.8, 55.9, 26.8, 26.1; MS (EI) m/z 265 (M^+), 248, 230, 206, 190, 136. Anal. Calcd for $C_{11}H_{15}N_5O_3$: C, 49.81; H, 5.70; N, 18.09. Found: C, 49.58, H, 5.74; N, 18.13.

9-[(1′*R*,2′*R*,3′*S*,4′*S*)-4′-Hydroxy-2′,3′-(isopropylidenedioxy)cyclohexanyl]adenine (29). To an ice-cooled mixture of **28** (265 mg, 1 mmol) and 2,2-dimethoxypropane (92 mL) in dry acetone (20 mL) was added 70% aqueous perchloric acid (0.25 mL). The reaction mixture was stirred for 2 h and then quenched with saturated aqueous $NaHCO_3$ (2 mL). The reaction mixture was concentrated, and the residue was adsorbed on silica gel (10 mg) and continuously extracted for 6 h with EtOAc (100 mL) using a Soxhlet apparatus. Evaporation of the solvent provided 260 mg (85%) of **29** as a white powder: mp 128–130 °C; IR (KBr) 3370, 3300, 3220, 3020, 2880, 1660, 1590, 1490, 1260, 1235, 1085 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.23 (s, 1 H), 8.12 (s, 1 H), 7.21 (s, 2 H, exchanged with D_2O), 5.23 (s, 1 H, exchanged with D_2O), 4.73 (m, 1 H), 4.41 (m, 1 H), 4.19 (br s, 1 H), 4.11 (br s, 1 H), 2.38 (m, 1 H), 1.78 (m, 2 H), 1.63 (s, 3 H), 1.59 (m, 1 H); ^{13}C NMR (DMSO-d_6) δ 156.0, 152.1, 149.5, 140.3, 119.2, 108.2, 78.4, 75.1, 64.9, 56.9, 28.1, 27.8, 26.2, 23.6; MS (EI) m/z 305 (M^+), 290, 230, 162, 136. Anal. Calcd for $C_{14}H_{19}N_5O_3$: C, 55.07; H, 6.27; N, 22.94. Found: C, 55.15; H, 6.23; N, 22.99.

9-[(1′*R*,2′*R*,3′*S*,4′*S*)-4′-Hydroxy-2′,3′-(isopropylidenedioxy)cyclohexanyl]-N^6-[1-(dimethylamino)ethylidene]-adenine (30). To a stirred solution of **29** (305 mg, 1 mmol) and molecular sieves (1 g, 3 Å) in dry dioxane (20 mL) was added *N*,*N*-dimethylacetamide dimethyl acetal (0.75 mL, 4.5 mmol). The reaction mixture was heated to reflux for 16 h. After concentration of the reaction mixture, the residue was chromatographed (CH_2Cl_2–MeOH, 99.5:0.5) to yield 330 mg (88%) of **30** as an amorphous powder: mp 110–112 °C; IR (KBr) 3440, 3020, 2960, 1620, 1590, 1570, 1440, 1355, 1235, 1085 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.57 (s, 1 H), 7.94 (s, 1 H), 4.76 (m, 1 H), 4.51 (m, 1 H), 4.37 (m, 2 H), 3.18 (br s, 6 H), 2.53 (m, 1 H), 2.16 (s, 3 H), 1.95 (m, 2 H), 1.85 (m, 1 H), 1.62 (s, 3 H), 1.34 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 161.3, 160.2, 152.4, 151.2, 140.8, 126.1, 109.4, 78.6, 76.2, 66.3, 57.7, 38.5, 38.3, 28.4, 28.1, 26.2, 23.9, 17.6; MS (EI) m/z 374 (M^+), 359, 304, 299, 204, 160, 134. Anal. Calcd for $C_{18}H_{26}N_6O_3$: C, 57.74; H, 7.00; N, 22.44. Found: C, 57.69; H, 7.05; N, 22.37.

9-[(1′*R*,2′*R*,3′*S*)-2′,3′-(Isopropylidenedioxy)-4′-cyclohexenyl]-N^6-[1-(dimethylamino)ethylidene]adenine (31). Compound **30** (187 mg, 0.5 mmol), upon treatment with DAST as described earlier for the preparation of **21**, afforded 160 mg (90%) of **31** as an amorphous powder: mp 70–72 °C; IR (KBr) 2980, 2820, 1600, 1570, 1390, 1330, 1215, 1060 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.53 (s, 1 H), 7.85 (s, 1 H), 6.04 (m, 2 H) 4.88 (m, 1 H), 4.72 (m, 1 H), 4.43 (m, 1 H), 3.18 (br d, 6 H), 2.56 (m, 1 H), 2.18 (s, 3 H), 2.02 (m, 1 H), 1.51 (m, 3 H), 1.35 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 161.1, 160.3, 152.4, 151.4, 141.4, 130.3, 126.7, 124.2, 109.6, 74.8, 72.6, 55.9, 38.5, 38.3, 29.5, 28.4, 25.7, 17.5; MS (EI) m/z 356 (M^+), 341, 298, 286, 204, 177, 149, 134. Anal. Calcd for $C_{18}H_{24}N_6O_2$: C, 60.66; H, 6.79; N, 23.58. Found: C, 60.58; H, 6.74; N, 23.63.

9-[(1′*R*,2′*R*,3′*S*)-2′,3′-Dihydroxy-4′-cyclohexenyl]adenine (3). Removal of protecting groups from compound **31** (178 mg, 0.5 mmol), as reported earlier for the preparation of **15**, yielded 86 mg (70%) of **3** after recrystallization from MeOH: mp 210–212 °C; IR (KBr) 3440, 3310, 3215, 2960, 2920, 1640, 1570, 1415, 1250, 1065 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.16 (s, 1 H), 8.08 (s, 1 H), 7.13 (s, 2 H, exchanged with D_2O), 5.77 (m, 2 H), 5.04 (s, 1 H, exchanged with D_2O), 4.68 (m, 1 H), 4.45 (s, 1 H, exchanged with D_2O), 4.20 (m, 1 H), 4.10 (m, 1 H), 2.79 (m, 1 H), 1.90 (m, 1 H); ^{13}C NMR (DMSO-d_6) δ 155.9, 151.8, 149.9, 140.9, 128.5, 127.3, 119.1, 69.6, 60.1, 52.2, 31.9; MS (EI) m/z 247 (M^+), 230, 211, 135. Anal. Calcd for $C_{11}H_{13}N_5O_2$: C, 53.44 H, 5.30; N, 28.32. Found: C, 53.56; H, 5.25; N, 28.43.

9-[(1′*R*,2′*R*,3′*S*)-2′,3′-Dihydroxycyclohexanyl]adenine (4). Catalytic hydrogenation of compound **3** (144 mg, 0.5 mmol) as reported earlier for the preparation of **16** yielded 140 mg, (95%) of **4** after recrystallization from MeOH: mp 270–272 °C; IR (KBr) 3310, 3250, 3180, 2930, 1640, 1595, 1410, 1050, 1040 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.14 (s, 1 H), 8.09 (s, 1 H), 7.12 (s, 2 H, exchanged with D_2O), 4.81 (br s, 2 H, exchanged with D_2O), 4.51 (m, 1 H), 4.10 (m, 1 H), 4.02 (m, 1 H), 2.07–1.41 (m, 6 H); ^{13}C NMR (DMSO-d_6) δ 155.8, 152.1, 149.9, 140.9, 119.2, 72.1, 69.3, 55.7, 31.3, 31.1, 18.9; MS (EI) m/z 249 (M^+), 232, 231, 204, 190, 177, 162, 148, 135. Anal. Calcd for $C_{11}H_{15}N_5O_2$: C, 53.00; H, 6.07; N, 28.09. Found: C, 53.12; H, 6.13; N, 28.01.

Purification of AdoHcy Hydrolase and Evaluation of the Effectiveness of Potential Inhibitors. AdoHcy hydrolase was purified from bovine liver as described,[19] except Q Sepharose (Pharmacia, Piscataway, NJ) was used instead of DE-52 cellulose and the CM-Sephadex step was omitted. The AdoHcy hydrolase activity was determined by the method of Richards et al.[20] which involves measuring the hydrolysis of [2,8-^{3}H]AdoHcy to [2,8-^{2}H]adenosine and homocysteine. The incubation medium contained 150 mM potassium phosphate, pH 7.6, and 1 mM EDTA. All incubations were performed at 37 °C. Different concentrations of potential inhibitors were preincubated with 20 nM AdoHcy hydrolase for 10 min. The preincubation mixtures were then incubated for 5 min with 4 units of calf intestinal adenosine deaminase and 100 μM [2,8-^{3}H]AdoHcy. The reaction was stopped by addition of 100 μL of 5 N formic acid, and the reaction mixture was applied to a column (1 × 4 cm) of SP Sephadex C-25 equilibrated in 0.1 N formic acid. The [2,8-^{3}H]inosine product of deamination of [2,8-^{3}H]adenosine (formed by the hydrolysis of AdoHcy) was eluted with 8 mL of 0.1 N formic acid. The eluate was collected and the radioactivity determined with 1 mL of eluate mixed with 10 mL of the scintillation cocktail (3a70, Research Products International, Mt. Prospect, IL) in a scintillation counter.

(20) Richards, H. H.; Chiang, P. K.; Cantoni, G. L. *J. Biol. Chem.* **1978**, *253*, 4478.

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Supplementary Material Available: Complete tabulated data from the NOE experiments performed on **9** and **29** as well as the COSY spectrum for **9** (3 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

Reagents for Bioorganic Synthesis. 5. The Synthesis of Two Potential Cross-Linking Reagents: 2,2′-Sulfonylbis[3-(benzylamino)-(*E*,*E*)-*N*-(2-oxoethyl)propenamide] (SBBOP) and 2,2′-Sulfonylbis[3-(benzylamino)-(*E*,*E*)-*N*-(2-chloroethyl)propenamide] (SBBCP)

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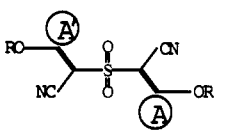
The syntheses of the title bifunctional organic reagents **3** and **4**, containing reactive bis(aldehyde) and bis(alkyl halide) functionalities, respectively, are reported. The reagents have potential applications for biomacromolecular cross-linking, in particular for cross-linking hemoglobin subunits.

One of our research objectives concerns the design and synthesis of novel mono-, bi-, and polyfunctional organic reagents for potential biomedical applications.[1] Our current focus is on bifunctional organic reagents for specifically cross-linking cell-free hemoglobins. The modified hemoglobins have potential use as blood substitutes for emergency transfusions.[2] The need for such an alternative is becoming increasingly pressing in view of scarcity of blood especially when rare types are needed, current limitations on storage of intact blood, the necessity for blood typing/cross-matching before transfusion, and the current public fear, in the wake of the AIDS epidemic, of possible transmission of blood-borne diseases including AIDS and hepatitis.

Cross-linking is anticipated to correct the two major problems associated with cell-free hemoglobin, which otherwise prevents its usage as a viable oxygen carrier: (1) the oxygen affinity of cell-free hemoglobin is too high to enable it to adequately deliver oxygen acquired from lungs to tissues and (2), outside of red blood cells, the tetrameric hemoglobin readily dissociates into α,β-dimers that are quickly eliminated by kidneys, causing hemoglobinuria.[3]

We have recently reported the synthesis, reactions, and applications of a few such bifunctional organic reagents (BORs).[1a-d] These BORs, as exemplified by reagents **1** and **2**, contained either bis(enol–ether)[1a,b,d] or bis(enamine)[1c] functionalities as the sites of cross-linking. Both are highly



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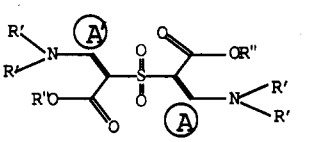

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electrophilic reagents and operate by initial conjugate addition of amine nucleophiles to their respective cross-linking sites (A,A′), followed by elimination of either alcohol or dialkylamine, producing stable secondary enamines as products. We have also demonstrated[1b,d] the versatility and high reactivity of **1** toward the building blocks of both proteins (amino acids) and nucleic acids (heterocyclic bases). Furthermore, we have shown reagent **1**'s utility in covalently cross-linking deoxy- and oxyhemoglobins.[1a] Likewise, reagent **2** was shown to undergo facile amine exchange reactions with a variety of primary amines.[1c] Nevertheless, the two reagents suffer from a couple of major drawbacks: one, their cross-linking tethers, as revealed by single-crystal X-ray diffraction analyses[4] of **1** (R = Me) and **2** (R′ = R″ = Me), are too short to make effective cross-links between the two diagonally opposed subunits (α_1 to α_2 or β_1 to β_2) of tetrameric hemoglobin, an essential characteristic sought in a cross-linker for

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